BIOLUMINESCENT DINOFLAGELLATES [1, 2]

BEATRICE M. SWEENEY [3]

Scripps Institution of Oceanography, University of California, La Jolla, California

From the days of the earliest voyages, men have observed the sparkling luminescence of the sea. That each fleck of light was usually the flash from a single creature of minute size rather than the phosphorescence of a chemical substance was known before the end of the eighteenth century (Harvey, 1952, 1957). The light-emitting properties of some of the dinoflagellates became apparent because these organisms were on occasion so very plentiful. One of the earliest to be recognized was *Noctiluca* (Baker, 1753; de Quatrefages, 1850) because of its large size and the great brightness of its luminescence, as well as its common occurrence along the shores of Europe. The "red tides" of *Gonyaulax polyedra* on the west coast of the United States and the extremely bright luminescence which accompanied them led to the identification of this organism as luminescent (Kofoid, 1911). A number of investigators have looked at luminescent sea water in the laboratory to try to identify the organism responsible (Dahlgren, 1924). The results of these studies have not always been definitive since the delicate organisms may have been in poor condition. Although culture of the dinoflagellates became possible following the discovery of their auxotrophy, observations of luminescence in cultures have seldom been reported, perhaps because most cultures were grown in continuous bright light, a condition which, in *Gonyaulax* at least, very much reduces the intensity of light emission. The relatively few species, the luminescence of which has already been well established, are given in Table I.

Since the author had at her disposal a photomultiplier photometer arranged for the measurement of the luminescence of the dinoflagellates (Sweeney, Haxo and Hastings, 1959) it seemed worth while to examine the common dinoflagellates of the coast of Southern California to determine which were luminescent and, perhaps equally interesting, which were not.

Materials and Methods

The dinoflagellates to be tested were collected from the ocean off Scripps Institution of Oceanography at La Jolla, California. Water samples were dipped from the surface. If the phytoplankton was not plentiful, the samples were concentrated by pouring the water through a plankton net or by removing a portion of

[1] Contribution from the Scripps Institution of Oceanography, New Series.

[2] This research has been supported in part by grants from the Marine Life Research Program of the California Cooperative Oceanic Fisheries Investigation and from The National Science Foundation.

[3] Present address: Department of Biology, Josiah Willard Gibbs Research Laboratory, Yale University, New Haven, Connecticut.

TABLE 1

Dinoflagellates previously reported to be luminescent

Genus and species	Reference
Ceratium tripos	Michaelis, 1830; Reinke, 1898; Zacharias, 1905
Ceratium furca	Ludwig, 1898
Gymnodinium flavum	Kofoid and Swezy, 1921
Gymnodinium sanguineum	Hirasaka, 1922
Gonyaulax catenella	Sommer *et al.*, 1937
Gonyaulax monilata	Connell and Cross, 1950; Howell, 1953
Gonyaulax polyedra	Torrey, 1902; Kofoid, 1911; Haxo and Sweeney, 1955
Gonyaulax polygramma	Nishikawa, 1901
Noctiluca miliaris	Baker, 1753; Michaelis, 1830; de Quatrefages, 1850
Peridinium candelabrum	Ludwig, 1898
Peridinium eugrammum	Ludwig, 1898
Peridinium granni	Ganapati *et al.*, 1959
Peridinium seta	Ludwig, 1898
Prorocentrum micans	Ehrenberg, 1834
Pyrocystis noctiluca	Murray, 1885
Pyrodinium bahamense	Plate, 1906

the water through a filter while retaining the plankton.[4] Dinoflagellates were isolated from these samples during the day and were washed once or twice in filtered sea water. Ten to twenty identical cells were placed in each of four test tubes. The contents of one were fixed with Rodhe's iodine fixative and set aside for the identification of the genus and species. The other three tubes were placed in the window in natural light. Since it is known that the luminescence of some dinoflagellates is rhythmic, being much brighter at night (Sweeney and Hastings, 1957), all testing for luminescence was done during the evening following isolation. Two of the three tubes from the window were tested for light emission, with the photomultiplier photometer recording intensity. Stimulation of luminescence was provided by a stream of air (Sweeney, Haxo and Hastings, 1959). The contents of the fourth tube, as well as that of the two tubes from which recordings had been made, were examined for living cells. The fourth tube was included because the aeration may fragment the fragile dinoflagellates during testing. This examination was important in cases where no luminescence was recorded, to make certain that living material had been examined and thus avoid falsely negative reports. Examination just prior to testing was impossible since the jarring contingent on microscopic examination might have stimulated luminescence prematurely, and recovery of the power to luminesce is sometimes very slow.

To supplement the data obtained from plankton samples, cells from laboratory cultures were also examined by the same technique.

RESULTS

Most of the dinoflagellates common in the phytoplankton at La Jolla between October, 1956, and May, 1959, were tested for luminescence. Cells came directly

[4] This method of concentrating phytoplankton samples, devised by Mrs. Anne Dodson of this laboratory, will be described in another publication.

from freshly collected water samples or were from cultures maintained in the laboratory. Some species were tested from both sources. The results of the study are presented in Table II. More than half of all the dinoflagellates tested proved to be non-luminescent. These included two species which had formerly been reported to emit light, *Prorocentrum micans* and *Ceratium furca*. Repeated tests of these species always gave negative results. All the species of *Gonyaulax* tested were luminescent. Some of these, like *G. polyedra* and *G. monilata,* were already known to be luminescent. Other genera represented contained both luminous and non-luminous species (*Ceratium* and *Peridinium*) or lacked luminous representatives (*Gymnodinium* and *Dinophysis*). In some cases, only a single species of a genus could be examined. It is interesting to note that while one culture of *Noctiluca* isolated from cells collected in the Gulf of California was

TABLE II

A. *Dinoflagellates found to be luminescent in this study*

Genus and species	Source*	Date tested
Ceratium fusus	S	10/18/56; 10/24/56; 11/8/56
Fragilidium heterolobum	S	3/20/57
Gonyaulax catenella	C	11/27/57
Gonyaulax hyalina	S	10/22/57
Gonyaulax monilata	C	11/27/57
Gonyaulax polyedra	C	1955–1963
Gonyaulax sphaeroidea	C	2/28/58
Noctiluca miliaris	C	1958–1959
Peridinium brochi	S	3/17/57
Peridinium conicum	S	3/10/58
Peridinium depressum	S	3/17/58
Peridinium pentagonum	S	5/7/57; 3/17/58

B. *Dinoflagellates found not to be luminescent in this study*

Ceratium dens	S	10/24/56; 4/11/57; 4/29/57; 5/7/57; 5/21/57; 5/11/59
Ceratium furca	S	11/8/56; 3/13/57
Dinophysis fortii	S	3/24/58
Dinophysis caudata	S	3/7/57; 3/24/58; 4/7/58
Dinophysis tripos	S	3/7/57; 3/24/58
Diplopeltopsis minor	S	5/26/58
Gymnodinium splendens	C	11/27/57
Noctiluca miliaris (?)	S & C	5/3/57
Peridinium claudicans	S	3/17/58
Peridinium subsalsum	C	11/27/57
Peridinium trochoideum	C	11/27/57
Prorocentrum micans	S	10/24/56; 4/9/57; 5/21/57
	C	11/27/57
Scrippsiella sweeneyi	C	11/27/57

C. *Diatoms found not to be luminescent*

Chaetoceros debilis	S	2/4/57
Coscinodiscus centralis var. pacifica	S	2/4/57
Ditylum brightwellii	S	2/4/57
Thallasiothrix mediterranea var. pacifica	S	2/4/57

* S designates cells isolated from the plankton at La Jolla; C, cells from cultures maintained in the laboratory.

brightly luminescent, two other cultures isolated from the La Jolla plankton were not luminescent. The cells of this *Noctiluca* were distinctly smaller even when they were growing rapidly in culture and the swarm spores were formed over an entire hemisphere of the cell rather than in a polar cap, as in the larger luminescent form, so that this may possibly be a different species (Eckert and Findlay, 1962).

Included for comparison are several diatoms from the phytoplankton. As expected, no diatom was found to emit light.

Indication of the reliability of the isolation and test methods was provided by the following observation. One sea water sample contained the following organisms in a 2-ml. aliquot:

Ceratium fusus	85
Ceratium furca	30
Prorocentrum micans	5
Chaetoceros (chains)	10
Ceratium sp.	2
Gyrodinium sp.	1
Silicoflagellate	1

The average luminescence emitted by such 2-ml. aliquots (10 samples) was 0.042 relative light units. Of the organisms found in this sea water, only *Ceratium fusus* tested positive for light emission, 20 cells yielding 0.0098 light units. This light emission is equivalent to 0.0416 light units from 85 cells. Thus, the light emitted by *Ceratium fusus* is sufficient to account for all the luminescence of the whole sample.

In general, then, many dinoflagellates belonging to a variety of general are able to emit light. However, by no means all species or even all genera possess this capacity.

The author wishes to express her gratitude to Dr. Enrique Balech and Dr. Robert W. Holmes for identifying the dinoflagellates in this study and to Mrs. Anne Dodson for technical assistance.

Summary

The common dinoflagellates of the San Diego region have been isolated and tested for the ability to emit light. The results are presented in the form of a table, Table II. All the species of *Gonyaulax* which were tested were luminescent. Other genera in which more than a single species was examined included at least some non-luminous members.

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